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A TWO-YEAR TEMPORAL AND SPATIAL INVESTIGATION OF QUESTING TICKS IN MARQUETTE COUNTY, MICHIGAN

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ABSTRACT

A TWO-YEAR TEMPORAL AND SPATIAL INVESTIGATION OF QUESTING TICKS IN MARQUETTE COUNTY, MICHIGAN

By

Tiffany J. Opalka

A two year investigation of the temporal and spatial distribution of questing ixodid ticks in Marquette County, Michigan during 2008 and 2009. The county was divided into two ranges, east and west using the county's natural geologic division. Two sites of each dominant habitat type (field, hardwood, mix, and conifer) were selected in each range (16 sites total). Four 100 meter transects were sampled weekly by dragging. Each time sampled, two of four plots at a site were randomly selected and within each chosen plot, two 100 meter transects were randomly selected. Season totals were 779 adult *Dermacentor variabilis* (421 females and 358 males) and 768 adult *D. variabilis* (381 females and 387 males) in 2008 and 2009, respectively. A single adult *Ixodes scapularis* was collected each year in the same field in the west. Chi-square analysis indicated significantly more ticks were collected in the east than west and significantly more ticks were collected in the field than the other habitats. Significantly more females were collected than males in 2008 but not in 2009. In 2008, questing tick abundance peaked late spring/early summer with a decline whereas in 2009, the peak occurred in mid spring. Habitat, week of sampling, and geologic range best predicted tick abundance based on multiple linear regression analysis.

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Introduction

Ticks are important vectors of human and animal diseases, with capabilities of transmitting a variety of infectious agents such as fungi, viruses and bacteria (Klompen et al., 1996; Sonenshine, 1991). The majority are non-nidicolous ticks that quest; i.e., purposefully ascend vegetation in order to come in contact with passing hosts (Sonenshine, 1993). This behavior increases the chance of humans to come in contact with ticks, which increases the risk of tick-borne diseases. Studying questing ticks to determine the composition, seasonal activity, and relative abundance of tick communities aids in understanding current risks of exposure to tick borne diseases (Sonenshine, 1993). In addition, information on existing tick communities can be used to predict the effect of global warming on tick distributions (Hartelt et al., 2008).

This two-year investigates the questing behavior of ticks in the geologically different eastern and western regions making up Marquette County, Michigan. In 2008 and 2009, four sites within each of four dominant habitat types (hardwood, field, deciduous and conifer mix, and conifer) were sampled once each week during spring, summer and early fall. The association of selected geologic and environmental characteristics with questing ticks was examined. Despite the utility of this information for estimation of disease risks and surveillance and control of ticks, only three studies have examined questing activity of ticks in the Upper Peninsula of Michigan and none specifically focused on Marquette County (Guerra et al., 2002; Hamer et al., 2007; Strand et al., 1992).

This study aimed to explore the species composition, temporal patterns and distribution of questing ticks in Marquette County, Michigan and to examine the relationship of their abundance with week of sampling, ambient temperature, relative humidity, habitat type, elevation, proximity to Lake Superior, and geologic features.

Tick information

General Tick Information

Ticks are ectoparasites of vertebrates that can be found throughout the world (Klompen et al., 1996). Three families make up the Order Ixodida: Ixodidae, Argasidae and Nuttalliellidae. Ixodidae or ixodid ticks are known as hard-bodied ticks because each has a hard chitinous scutum. This is the largest tick family, comprised of 13 genera and about 694 species (Parola & Raoult, 2001; Sonenshine, 1991). Most Ixodidae are non-nidicolous ticks and seek their hosts by questing on vegetation. The remaining ixodid species are nidicolous and seek hosts by waiting in nests or burrows of their hosts (Sonenshine, 1993). Argasidae ticks are called soft bodied because each lacks a dorsal shield or scutum. Although some quest for hosts on vegetation, most are nidicolous and are found in nests or burrows (Klompen et al., 1996). This family is comprised of 5 genera and about 177 species (Parola & Raoult, 2001). Nuttalliellidae shares morphologic characteristic with both Ixodidae and Argasidae and consists of a single southern Africa species (Klompen et al., 1996; Parola & Raoult, 2001).

Non-nidicolous ixodid ticks quest for hosts in habitats such as meadows, forest floors, soil, and brush (Klompen et al., 1996; Sonenshine, 1991). Questing non-nidicolous ticks are exposed to fluctuations in ambient temperature and relative humidity that can cause dehydration. When this happens ticks can protect themselves by retreating into the leaf litter, an environment with higher humidity (Sonenshine, 1993).

There are four life stages in ticks of Ixodidae: egg, larva, nymph and adult (Sonenshine, 1991). After mating, an engorged adult female can lay several thousand eggs at one time, and once her egg clutch is laid she dies. Under appropriate conditions the eggs develop and hatch into six-legged larvae (nymphs and adults have eight legs). There is only one nymphal instar associated with this family, and there is no morphological sex differentiation until the adult stage (Klompen et al., 1996). The size of the scutum is different depending on sex and life stage. In adult males, the dorsal surface of the body is completely sclerotized whereas in adult females and immature life stages, only the anterior portion of the dorsal surface is sclerotized to allow for greater expansion when taking large blood meals.

Each ixodid tick species follows one of three life cycles: 1-host, 2-host, or 3-host (Oliver, 1989). One-host ticks quest in the larval stage and once they find a host, they remain on that host until they become adults. After the adults mate, the engorged females drop off the host to lay their eggs. Thus, only the larval stage would be found questing. Two-host ticks quest as larvae, feed and then molt into nymphs on the same host. After the nymphs feed, they drop off to molt into adults who then quest for a second host where they feed and mate. After engorgement, females fall off to lay eggs. These ticks have two stages that would be found questing: larval and adult stages. The 3-host life cycle is the most common (Sonenshine, 1991). The larvae quest for their first host, feed and drop off. They molt into nymphs and quest for their second blood meal. Once they have fed, they drop off and molt into adults. After successfully questing for the third host, they feed and mate to complete the life cycle. All three life stages (larval, nymphal, and adult) would be found questing.

Seasonal activity

Two types of diapause occur in ticks (Sonenshine, 1993). Morphogenetic (developmental) diapause is when ticks delay their development (embryogenesis, ecdysis of engorged immatures, and oviposition) for a specific period of time. Once ticks enter this diapause, they cannot reverse it even under good environmental conditions. Behavioral diapause is the cessation of host-seeking in unfed ticks and delayed engorgement in attached ticks. This occurs when energy reserves have been exhausted or temperature/relative humidity become less desirable and doing so increases survival (Harlan & Foster, 1990; Sonenshine, 1993). The timing of diapause is highly dependent on the species' geographic range (McEnroe, 1985). The length of photoperiod allows populations to synchronize their host-seeking activity and feeding behavior with favorable climatic conditions (Sonenshine, 1993). The effect of photoperiod varies with tick species. For example, seasonal activity for *Dermacentor variabilis* increases with increasing photoperiod, whereas seasonal activity for *D. albipictus* increases with decreasing photoperiod (Atwood & Sonenshine, 1967). In sub-tropical regions of the United States, high temperatures and the resulting decreased moisture availability to ticks are also important in regulating the timing of diapause. In these regions, species such as *Ixodes scapularis* have adapted by entering diapause in the summer. Their activity occurs during fall and winter, thereby reducing the exposure of free-living stages to lethal temperatures. In temperate and subarctic regions, *I. scapularis* is active during summer conditions (milder temperatures and more available moisture) and enters diapause during fall and winter (McEnroe, 1985). In addition, snow cover and reduced sunlight in this region limit questing activity. Behavioral and morphogenetic diapause patterns foster thriving tick populations during optimal climatic conditions (Sonenshine, 1993).

Some species such as *D. variabilis* can have a 1-year or 2-year life cycle depending on the region they inhabit. The 1-year life cycle is more common in sub-tropical regions where sunlight levels and temperatures favor tick activity year round (McEnroe, 1985; Sonenshine 1993). A 2-year life cycle is typical for temperate and subarctic regions (Figure 1; Sonenshine, 1993). In the fall, unfed ticks (larvae, nymphs, and adults) enter diapause during undesirable winter conditions and emerge in the spring to commence questing. When seasonal activity begins in the spring, the population consists primarily of unfed larvae and adults from the previous fall (Sonenshine, 1993). Overwintering survival of the nymphal stage in these regions is markedly lower than that of larval or adult stages for reasons not completely understood (Sonenshine, 1972; Sonenshine, 1993). During the first year of the life cycle, the overwintered larvae emerge in the spring to quest and larval abundance declines as individuals either find hosts or die. As some immature ticks become adults, a seasonal peak of questing activity occurs. After mating and feeding, engorged females lay their eggs and die. The eggs hatch in late summer or early fall, and the majority of these larvae enter diapause until the following spring. But many larvae and nymphs take much longer to find hosts and to molt. As a result, they enter diapause as nymphs or adults for winter. Thus, the life cycle is not completed until the second year.

Surveys of *D. variabilis* have documented a 2-year life cycle in temperate and sub-arctic regions based on peaks in questing activity. In Nova Scotia, Canada, adults were found questing from April to August, with a peak of newly molted adults occurring in May or June, evidence that the peak is comprised of overwintered immatures becoming adults (Garvie et al., 1978). In Ohio, seasonal activity of adults commenced in April and continued into September with a peak of questing adults in May or June. A second, smaller peak in August or September can occur if adults do not enter diapause (Conlon & Rockett, 1982).

Survey methods

Most tick studies use one or more of three common sampling methods: trapping and examining hosts, tick dragging, and dry ice (CO_2) baited tick traps (Falco & Fish, 1992). Two of the three techniques (tick dragging and CO_2 baited traps) detect questing stages and they vary in their efficiency for collecting particular species and stages (Ginsberg & Ewing, 1989; Petry et al., 2010; Sonenshine, 1993). However, Falco & Fish (1992) determined that tick dragging is the most effective method for detecting questing ticks, and dragging is commonly used for sampling questing ticks (Daniels et al., 2000; Petry et al., 2010; Sonenshine, 1993; Yu et al., 2011). Tick dragging consists of dragging a 1m x 1m white flannel cloth with one end attached to a wooden dowel and the other end weighted to increase its contact with the vegetation. The cloth is dragged along transects and is checked periodically for ticks to reduce tick drop-off.

Environmental factors that influence tick survival

Two and 3-host ticks spend about 95% of their life off-host digesting blood meals, molting, and questing. Thus, off-host sampling in habitats is a valuable method for studying tick communities (Klompen et al., 1996; Needham & Teel, 1991; Oliver, 1989).

The suitability of the habitat is determined by the tick's ability to avoid desiccation, excessive moisture, predators, and parasites (Sonenshine, 1993; Stein et al., 2008).

Major factors (primarily temperature and relative humidity) that determine the geographic distribution of ticks can be measured at three scales: macroclimate, mesoclimate, and microclimate (Sonenshine, 1993). The macroclimate scale examines the general climatic conditions of a broad geographic area. Based on conditions at this scale, the general range of tick populations can be estimated. For example, close proximity to a large body of water like Lake Superior increases relative humidity and decreases temperature extremes (Stein, 2001). The scale at which questing ticks experience their environment is the mesoclimate. This is the area within the vegetative layer and below the canopy (e.g., the grass and weeds, small trees and bushes of a habitat). Mesoclimate conditions differ from the macroclimate by having less extreme temperatures and higher humidity. The climate most important to free-living ticks is the microclimate within the soil and leaf litter, and it is often very different from the mesoclimate and macroclimate. At this scale, temperatures are less extreme and humidity is higher than both mesoclimate and macroclimate. The microclimate provides a nearly saturated relative humidity refuge that allows ticks to restore body water. However, the full set of factors that make the microclimate favorable are incompletely known and difficult to assess. Ambient temperature at the mesoclimate scale is a good predictor of tick questing behavior likely because of its effect on humidity and water balance in ticks (Atwood & Sonenshine, 1967; Harlan & Foster, 1990; McEnroe & McEnroe, 1973; Schulze & Jordan, 2005). In low humidity conditions, ticks spend less time questing and more time in the more humid leaf litter (Harlan & Foster, 1990; Sonenshine, 1993).

Climate or the weather pattern influences tick survival during diapause as well as during molting and questing because of its impact on ambient temperature and relative humidity. In Canada and midwestern states (Illinois, Michigan, Minnesota, Ohio, and Wisconsin), abrupt temperature fluctuations during seasonal changes from winter to spring, as well as large amounts of snowfall affect tick activity. During unusually warm temperatures in winter, some ticks come out of behavioral diapause to commence questing activity. If temperatures return to the usual range, they stop questing and seek shelter in soil/leaf litter. McEnroe & McEnroe (1973) reported that the more times a tick starts and stops questing, the higher their mortality.

Soil and vegetation are important refuges for free-living ticks. During winter, ticks retreat to soil for protection from the harsh climate. An insulating layer of snow provides protection from extreme temperatures. A lack of snow can cause tick death because exposure to ice can rupture cells. Exposure to low temperatures reduces the tick's ability to regulate water, and high humidity during winter can cause ice formation on ticks from condensed water vapor (Burks et al., 1996; McEnroe, 1975). Ambient temperatures during spring, and levels of radiant heating with the increase of the azimuth of the sun, regulate initial spring activity of ticks (McEnroe, 1978).

Elevation varies in its impact on the tick community. For example, established populations of *I. scapularis* occurred on white-tailed deer (*Odocoileus virginianus*) in all areas of Maryland except the western part which is higher in elevation and colder compared to the rest of the state. Highest abundance of *I. scapularis* was found in the coastal plains (Amerasinghe et al., 1992). In New Jersey and Maine, a similar pattern was seen for *I. scapularis* while *D. albipictus* was found in low and high elevations (Rand et al., 2003; Schulze et al., 1984). In Colorado, adult *D. andersoni* abundance showed a parabolic relationship with elevations between 2100 meters to 2500 meters (Eisen et al., 2007).

Marquette County's tick population

The most frequently detected ixodid tick species in Michigan are *Amblyomma americanum*, *D. albipictus*, *D. variabilis*, *Haemaphysalis leporispalustris*, *I. cookei* and *I. scapularis* (Walker et al. 1998). These species vary in preferred habitat, number of hosts needed to complete life cycle, preferred host, and questing behavior (nidicolous or non-nidicolous) (Table 1). For five of these six species, at least one stage quests, and thus would be detectable where present in Marquette County.

Amblyomma americanum, the lone-star tick, is capable of transmitting *Ehrlichia chaffeensis,* causative agent of human monocytic ehrlichiosis (Childs & Paddock, 2003). This southern tick is expanding its range northward and is rarely reported in Marquette County (Ginsberg et al., 1991; Walker et al., 1998). *Amblyomma americanum* has a 3-host life cycle and thus all three feeding stages will quest. *Dermacentor albipictus,* the winter tick, is well established in Marquette County and it has a 1-host life cycle, and thus only larvae quest and only in the fall (Lawrence et al., 1965; Sonenshine, 1993; Walker et al., 1998). Bacteria belonging to the genera *Anaplasma* and *Francisella* have been transmitted from female *D. albipictus* to her offspring but its ability to transmit to humans is unclear (Baldridge et al., 2009) Also, *Borrelia burgdorferi* has been detected in *D. albipictus* but it has not been shown to transmit to humans (Baldridge et al., 2009). *Dermacentor variabilis,* the American dog tick, is a 3-host tick found in high abundance in Marquette County (Bishop & Trembley, 1945; Walker et al., 1998). This tick can transmit *Rickettsia rickettsii,* which causes Rocky Mountain spotted fever (RMSF) and *Francisella tularensis* which causes tularemia (Goethert et al., 2004; Wikswo et al., 2008). Little is known about the abundance of the 3-host tick *H. leporispalustris,* the rabbit tick, in the Upper Peninsula of Michigan (Walker et al., 1998). However, one specimen was collected from a dog in Marquette County in 2009 (J. Bird, Personal Communication, 2011). *Rickettsia rickettsii* has been found in *H. leporispalustris,* and although this tick does not bite humans it does play a role in maintaining and spreading RMSF. *Dermacentor variabilis,* the tick vector of RMSF, acquire the rickettsia when immature stages feed on infected rabbits. The tick remains transtadially infected. The rickettsia may be transmitted when these newly molted infected *D. variabilis* feed on other mammals (Hun et al., 2008; Sonenshine, 1993). *Ixodes cookei,* in some areas is known as the groundhog tick, has a 3-host life cycle, and is capable of transmitting the virus that causes Powassan encephalitis in humans (Ebel, 2010). This tick has been found in Marquette County in varying abundances by mammal trapping and because it is nidicolous, it is not found questing (Lawrence et al., 1965; Walker et al., 1998). The final species *I. scapularis,* the deer tick, is a 3-host tick. It is capable of transmitting at least three pathogens to humans: *B. burgdorferi,* the causative agent of Lyme disease, *Anaplasma phagocytophilum,* the causative agent of human granulocytic ehrlichiosis, and *Babesia microti,* the causative agent of human babesiosis (Holman et al., 2004). Most recently, an unnamed ehrlichia species has been recovered from humans and *I. scapularis* in Minnesota and Wisconsin (Pritt et al., 2011). Abundance of *I. scapularis* is poorly studied in the Upper Peninsula. However, its presence in Marquette County is highly probable based on the following evidence.

- *Ixodes scapularis* is abundant in Menominee County, located directly south of Marquette County (Guerra et al., 2002; Strand et al., 1992; Walker et al., 1998).
- There was a single unconfirmed report of *I. scapularis* collected from a human and a second confirmed report collected from a dog in Marquette County in 2006. Most recently, two male *I. scapularis* and one nymph were collected in Marquette County in 2011.
- In a three year period, 2003-2005, three feeding ticks were found on trapped fishers (*Martes pennanti)* in Alger County, an adjacent county (J. Bird, Personal Communication, 2008).
- Areas in Marquette County have environmental conditions that give higher than 50% probability of establishment (Brownstein et al., 2003)

Marquette County Geography

Upper Peninsula and Marquette County geology

The Upper Peninsula can be divided into a western half and an eastern half based on geologic categories. The western half is a southern extension of the Canadian Shield; it is made up of Precambrian and Cambrian rocks dating back 600 million to 4.5 billion years (Heinrich, 1976). This half of the Upper Peninsula includes the Huron Mountains and Porcupine Mountains, with terrain consisting of rugged, steep ridges and hills. Elevations range from 184 to 604 meters (Albert, 1995; Gair & Thaden, 1968; Heinrich, 1976). The eastern half of the Upper Peninsula consists of rocks from Ordovician and Pennsylvanian eras dating to approximately 300 million years ago (Heinrich, 1976). This area has less rugged terrain and a larger portion of wetland area than the western half and elevations ranging from 177 to 395 meters (Gair & Thaden, 1968; Heinrich, 1976). However, similar habitat types are found in both halves (Michigan Geographic Data Library Center for Geographic Information, MiCGI, 2001). Marquette County is situated where these geologic differences separate the county into a western and an eastern range (Figure 2).

Marquette County's dominant habitat types

Marquette County is located in the north central region of the Upper Peninsula of Michigan and borders Lake Superior to the north. The county has a total area of 8,871 km^2; 4,716 km^2 of it is land and 4,155 km^2 of it is water (U.S. Census Bureau, 2000). Marquette County can be classified into five different landscape ecosystems based on abiotic (climate, elevation, geology, and glacial formations) and biotic (soils, vegetation, and animals) characteristics (Figure 2) (Albert, 1995; Smith, 2002).

Western landscape classifications

IX.2. Michigamme Highland. This subsection is composed of Precambrian rock with hardwoods such as red oak (*Quercus rubra)*, sugar maple (*Acer saccharum*), yellow birch (*Betula alleghaniensis*), and conifers such as white pine (*Pinus strobus)*, red pine (*Pinus resinosa)*, and some localized jack pines (*Pinus banksiana)*. Elevations range from approximately 184 meters to 604 meters. This subsection contains the highest points in Marquette County.

IX.3.2. Winegar Moraine. This area contains steep sandy ridges, wetlands, and lakes. Elevations range from approximately 389 meters to 563 meters. There are hardwoods composed of sugar maple, yellow birch, red maple (*A. rubrum*), and basswood (*Tilia americana*) in the higher elevations, and there are some bogs with hemlock (*Tsuga canadensis*), black spruce (*Picea mariana*) and tamarack (*Larix laricina*) swamps in this subsection.

Eastern landscape classifications

VIII.3.3. Deerton. This subsection is composed of sandstone and high sandy ridges with hardwood forests and conifer swamps that include hemlock, white pine, white cedar *(Thuja occidentalis)*, and spruce *(Picea* sp.). Elevation ranges from approximately 185 meters to 395 meters.

VIII.3.2. Gwinn. The land primarily consists of sandy plains which are either poorly drained or excessively drained. The poorly drained wetlands are composed of swamps, which include balsam (*Abies balsamea*), white cedar, spruce, and tamarack. The excessively drained areas contain jack pine stands. Elevation ranges from approximately 314 meters to 378 meters.

VIII.3.1. Northern Lake Michigan (Hermanville) Till Plain. Wetlands are extensive throughout this subsection with white cedar swamps and mixture of hardwood-conifer swamps. Examples of hardwood are beech (*Fagus grandifolia*), yellow birch, elm (*Ulmus* spp.), maple (*Acer* spp.), and oak (*Quercus* spp.). Some examples of swamp conifers are balsam, cedar, spruce, and tamarack. There are small hills approximately 45 meters high, called drumlins, these make up the till plain and the overall elevation ranges from approximately 177 meters to 381 meters.

Marquette County's climate

The winter months in Marquette County can be harsh with a monthly average of -9.7 degrees Celsius during December, January, and February and average monthly precipitation of 5.8 cm. During spring (March, April and May), temperatures rise and fall frequently with frost events occurring in May. The spring average was 2.7 degrees Celsius and the monthly average precipitation was 7.6 cm. The average for summer (June, July and August) was 16.7 degrees Celsius and monthly average precipitation was 8.3 cm. All averages were based on 30-year monthly averages collected by National Climatic Data Center (NCDC)/National Environmental Satellite, Data, and Information

Service (NESDIS)/National Oceanic and Atmospheric Administration (NOAA) (2002) (Table 2).

The winter of 2008 (December 2007, January 2008, and February 2008) average was -9.3 degrees Celsius, and monthly average precipitation was 1.2 cm (Table 2). For spring 2008 (March, April and May), the monthly average was 1.4 degrees Celsius and the monthly average precipitation was 8.9 cm. The summer of 2008 (June, July, and August) average temperature was 17.0 degrees Celsius and a monthly average precipitation was 5.6 cm. During the winter of 2009, the average was -10.3 degrees Celsius and average precipitation was 6.9 cm. For spring 2009, the monthly average was 2.7 degrees Celsius and average precipitation was 5.8 cm. For the summer of 2009 the average was 15.0 degrees Celsius and the average monthly precipitation was 7.2 cm. All averages were calculated using data from a single weather station (National Weather Service Weather Forecast Office for 2008 and 2009, NWS, 2011) (Table 2).

The winter temperatures for 2008 and 2009 were similar to the historic averages. However, the 2008 snow fall was less and 2009 snow fall was slightly more than historic averages. Compared to historical averages, 2008 spring was cooler and wetter whereas 2009 had the same spring temperature as historic average but was drier. The 2008 summer was warmer and drier while the 2009 summer was cooler and drier compared to historic averages (Table 2).

Study Objectives

The main objective of my study was to determine the human risk of exposure to ticks in Marquette County, Michigan. I did this by dragging for questing ticks, estimating the species composition, and describing their temporal and spatial distributions with respect to the following selected factors: week of sampling, ambient temperature, relative humidity, geologic range, elevation, habitat, and distance from Lake Superior. The expected associations between questing ticks and these factors were:

- Week of sampling: abundance of questing ticks over time will show a unimodal pattern for this region.
- Ambient temperature and relative humidity: as temperature increases and humidity decreases, fewer questing ticks will be detected.
- Geologic range and elevation: as elevation increases, tick abundance decreases. Thus, lower tick abundance in west than in east.
- Habitat type: in all habitats with leaf litter, questing ticks will be detected.
- Distance from Lake Superior: as distance to Lake Superior decreases, questing tick abundance will increase.

Materials and Methods

Habitat type descriptions

Four habitat types that dominate Marquette County were chosen: hardwood, field (herbaceous open-land), deciduous and conifer mix, and conifer (Albert, 1995; MiCGI, 2001; Smith, 2002). These four habitat types were defined as (1) northern hardwood forests: more than 60% of the trees were hardwood trees (e.g., sugar maple, red maple, basswood, yellow birch, elm, beech, cherry (*Prunus* sp.), white ash (*Fraxinus americana)*; (2) herbaceous open-land areas: less than 25% of the land area consisted of woody canopy cover (e.g., prairies, grasslands, rangelands, power line tracts, agricultural croplands); (3) deciduous and conifer mixed forests: there were not less than 40% nor more than 60% of hardwoods or conifer; (4) conifer forests: more than 60% of the trees were conifer trees (e.g., red (*Pinus resinosa)*, white (*Pinus strobus)*, jack pine (*Pinus banksiana)*) (Michigan Department of Natural Resources, MDNR, 1991).

Site selection and locations

Two sites of each dominant habitat type were selected in the east and in the west ranges of Marquette County (16 sites total). Preliminary selection of these sites was based on a land cover/use map from the Michigan Center of Geographic Information, and landscape ecosystem classifications by the United States Geological Survey Northern Prairie Wildlife Research Center (Figure 2; Albert, 1995; MiCGI, 2001). Once a site was selected, it was ground-truthed to confirm that the desired habitat was present and large enough to include four contiguous 100 meter x 100 meter plots. Accessibility and permission to use were also determining factors in site selection. Access was granted from the MDNR for state property and from private land owners for the remaining sites.

During late April and into early May of 2008, the sites were selected and staked out. A Global Positioning System receiver (GPS) was used to obtain coordinates and elevations of each site. In 2009, all sites remained the same except an alternate conifer site was used in the west due to logging of the 2008 site (Figure 3).

Sampling and dragging procedures

All 16 sites were sampled once per week. Sampling occurred during one of two periods: 0800 - 1200 or 1300 - 1700. For each site, the time period alternated weekly (i.e., if the site was sampled during the morning one week, it was sampled in the afternoon the following week). If it was raining, the site was sampled at the designated sampling time on a different day of that week. In 2008, sampling began May 18 and ended August 16, a total of 12 sampling weeks. In 2009, sampling began May 4 and ended August 22, a total of 16 sampling weeks.

On the day of sampling, two of the four plots at a site were randomly selected and within each chosen plot, two 100 meter transects were randomly selected to determine where to begin dragging east of the southwestern corner of the plot (Figure 4). Thus, 400 meters were dragged each time a site was sampled. The random numbers generated were used for all sites surveyed that day.

Once a starting point was determined, a 1 meter x 1 meter white flannel cloth was dragged from south to north along each 100 meter transect, stopping every 10 meters to remove ticks from the cloth. Efforts were made to drag the cloth at a constant pace over the vegetation so the relative number of ticks collected could be compared throughout the survey. All transects were traversed in a straight line as the terrain allowed. Each sample effort took approximately 90 minutes. All ticks collected from each site were placed in a labeled vial containing 70% ethanol with 5% glycerin. The ticks were brought back to the laboratory and identified to species, stage, and sex.

Factors of Importance

Historical macroclimate data (1971-2000) was obtained from NCDC/NESDIS/NOAA (2002). The macroclimate data for 2008 and 2009 was obtained from National Weather Service Weather Forecast Office (2011). The following information was recorded for each site: habitat type, range (east or west), GPS coordinates, elevation, and distance from Lake Superior. For each site, GPS coordinates were entered into Google Earth (2010) and the shortest distance from that point to Lake Superior was calculated. HOBO ProV2 data loggers were used at 11 sites during the 2008 sampling period and all 16 sites during 2009. The data loggers were placed in easily accessible spots about 61 cm above ground (in the mesoclimate) at each site and remained there throughout the season. They were exposed to sun and shade as the day progressed. Ambient temperature and relative humidity were recorded at 15 minute intervals and the logger was kept in the same position throughout the survey. Data were downloaded from the logger onto a personal computer at the end of each season. Average ambient temperature and relative humidity were calculated based on the 90 minute time-frame during which sampling occurred.

Data Analysis

All statistical tests were run using Statistical Package for the Social Sciences, PASW Statistics 18 (PASW, 2009) with $p \leq 0.05$. General loglinear analysis was performed to examine the relationships among the main factors (range, habitat and sex). If no significant 2-way or 3-way interactions were present, then Chi-square analyses were run to determine whether the total abundance of questing ticks differed within the main factors: range, sex, and habitat. Yate's continuity correction was used when performing Chi-square analyses with one degree of freedom (Norusis, 2006).

The tick season was divided into 3-week periods: mid-spring (May 3 – May 24), late spring (May 25 – June 14), early summer (June 15 – July 5), mid-summer (July 6 – July 26), and late summer (July 27 – August 16). The numbers of ticks collected during each 3-week period of each year was graphed as 3-week moving averages to show temporal patterns. Because sampling began later in 2008, mid spring data were not available for that year. If peak activity appeared to be at different times and/or tick numbers were dramatically different, its association with climate factors (temperature, snow depth and precipitation) was investigated. Daily measurements of these factors were available from a single weather station in Marquette County and were graphed against winter and spring months of 2008 and 2009 (NWS, 2011).

A stepwise multiple linear regression was run to examine the relationship between selected variables and tick abundance. Because tick abundance did not meet the assumptions of a linear regression, these data were square root transformed. In 2009, these variables were: week of sampling, ambient temperature, relative humidity, range (west or east), site (1 or 2), elevation, habitat type (field, hardwood, mix, or conifer) and distance from Lake Superior. When analyzing 2008 data, I used the same variables as for 2009 except ambient temperature and relative humidity.

Results

Species composition and temporal pattern

Of the 1549 ticks collected during this study, 1547 were adult *D. variabilis*. A single adult *I. scapularis* was collected each year from the same field in the western half of Marquette County. Season totals were 779 *D. variabilis* (421 females and 358 males) and 768 *D. variabilis* (381 females and 387 males) in 2008 and 2009, respectively (Table 3). Eastern range totals were 552 and 616 *D. variabilis* and western range totals were 227 and 152 *D. variabilis* for 2008 and 2009, respectively. The same pattern in tick abundance among habitats was observed in 2008 and 2009: field had highest abundance (463 ticks and 594 ticks), hardwood next highest (218 ticks and 131 ticks), mix third highest (98 ticks and 41 ticks), and conifer had only 2 ticks in 2009 (Figure 5).

Loglinear analysis showed no significant two- or three-way interactions among range, sex and habitat, indicating that the effects of these variables on tick abundance were independent of each other. In both 2008 and 2009, significantly more ticks were collected in the eastern part of Marquette County than in the western part ($\chi^2{}_{'08}$ = 135.591, $p \leq 0.001$, $df = 1$ and $\chi^2{}_{'09}$ = 280.333, $p \leq 0.001$, $df = 1$). Also, significantly more females (421) were collected than males (358) in 2008 ($\chi^2 = 5.095$, $p = 0.024$, $df = 1$) but not in 2009 (females: 381; males: 387) ($\chi^2 = 0.047$, $p = 0.829$, $df = 1$). Significantly more ticks were collected in field habitat than in hardwood, mix and conifer ($\chi_{'08}$ = 266.560, p = 0.000, $df = 2$ and $\chi_{'09}$ = 1167.844, $p = 0.000$, $df = 3$).

Questing tick abundance

In both 2008 and 2009, the number of questing adult ticks exhibited a unimodal pattern (Figure 6). In 2008, questing tick numbers peaked in early summer, and then declined. During the 2009 season, sampling began three weeks earlier than in the 2008 season and there were already a high number of ticks questing. Following the mid spring peak, numbers gradually declined.

Multiple linear regression analysis of the more complete 2009 data set indicated that of the 13 variables, only field, week of sampling, and range were significantly associated with tick numbers. The model indicated that field habitats had more ticks than the other three habitat types, fewer ticks were collected as the sampling season progresses, and more ticks were collected in the eastern half of Marquette County compared to the western half. The linear equation was:

$$\sqrt{(\text{tick abundance})} = 1.245 + 0.390\ (\text{Field}) - 0.370\ (\text{Week}) + 0.189\ (\text{Range})$$

However, the model did not explain much of the variability in the data ($R^2 = 0.325$, $F_{3,\ 220} = 35.333$, $p \leq 0.001$).

Multiple linear regression of 2008 data used the same variables but without ambient temperature and relative humidity produced a remarkably similar equation.

The linear equation was:

$$\sqrt{(\text{tick abundance})} = 3.078 + 0.291\ (\text{Field}) - 0.313\ (\text{Week}) + 0.220\ (\text{Range})$$

But again the model did not explain much of the variability in the data ($R^2 = 0.213$, $F_{3,\ 71} = 6.400$, $p = 0.001$).

Discussion

Species composition

Dermacentor variabilis overwhelmingly dominated the questing tick community in this study. They were collected from all 16 sites and only adults were collected. Interestingly, more questing female *D. variabilis* were collected than male during 2008 sampling but there was no significant difference between the sexes in 2009. Conlon and Rockett found more questing male *D. variabilis* than female during their 2-year study in Ohio (1982). These varied results suggest that questing responses to environmental conditions may differ between sexes.

My study indicates that hosts in Marquette County are exposed almost exclusively to questing *D. variabilis* with a low risk of picking up *I. scapularis*. Two studies that studied Michigan's tick community reported higher species richness than I found in my study (Lawrence et al., 1965; Walker et al., 1998). Lawrence et al.(1965) collected ticks off live and recently dead mammals over 2 years and found eight tick species. Walker et al. (1998) examined ticks submitted by citizens over a 12 year period and ticks found on hunter-killed white-tailed deer over a 3 year period. They reported 21 species of ticks. These studies sampled feeding ticks (both nidicolous and non-nidicolous) rather than questing ticks (non-nidicolous) over a broader geographic range and over more tick seasons. The low species richness of questing ticks is a result of my focus on non-nidicolous species that quest in Marquette County.

The absence of immature stages is a common problem in tick surveys that use drags. Although they are the most abundant stage, free-living larvae are highly aggregated (Sonenshine & Levy, 1972). This results in small pockets of questing larvae and large areas of habitat with no questing larvae. In addition, because larvae quest for their preferred hosts near the base of vegetation or on leaf litter-detritus layer, it is difficult for the drag cloth to contact them (Campbell & MacKay, 1979; Garvie et al., 1978; Sonenshine & Levy, 1972). Free-living nymphs are less abundant, but more abundant than adults. They tend to quest at a higher height than larvae, but at a lower height than adults. In contrast, free-living adults, although least abundant, are the least aggregated which results in increased capture by drag cloth. Adult ticks quest for their larger hosts (e.g., white-tailed deer, bear (*Ursus* spp.), humans) at higher heights (Dodds et al., 1969; Schulze et al., 1984). Ticks questing at higher heights are detected more easily through dragging (Sonenshine, 1993; Sonenshine & Levy, 1972). The absence of immature *D. variabilis* in my study likely reflects the difficulty in detecting them by dragging in an environment where low plant growth prevents the drag from contacting questing ticks. This is similar to what other studies found (Ginsberg & Ewing, 1989; Perla & Thomas, 2001).

Questing tick abundance

Seasonal questing activity of adult *D. variabilis* followed a unimodal pattern but the peaks occurred at different times in each year (Figure 6). A unimodal pattern is consistent with those seen in other studies on *D. variabilis* in temperate and sub-arctic regions (Conlon & Rockett, 1982; Garvie et al., 1978; McEnroe, 1979). Questing adult tick numbers peak when overwintered larvae and nymphs have fed and molted into adults

(Sonenshine, 1993). When overwintered ticks can commence questing affects when the questing activity peaks for each year. It is likely that a later peak occurs when unfavorable spring conditions delay questing by overwintered ticks.

Spring related factors may have delayed tick questing in spring of 2008 compared to 2009. Snow depth data from a single weather station in Marquette County suggested that in 2008 snow cover prevented questing until April 22 while in 2009 a lack of snow cover allowed questing by April 14 (Figure 7; National Weather Service Forecast Office, NWS, 2011). In addition, warmer temperatures during this time of little to no snow would have promoted questing and tick development, shortening the length of the life cycle.

The lack of influence of several selected variables on *D. variabilis* abundance in Marquette County was unexpected. Close proximity to Lake Superior decreases temperature extremes and increases relative humidity (Stein, 2001), both favorable for free-living ticks. However, distance from Lake Superior was not negatively associated with tick abundance likely because questing ticks can be protected from these extremes by entering the leaf litter/soil component of their habitat. Increasing elevation tends to decrease temperature and moisture availability, and tick species vary in their responses to elevation (Amerasinghe et al., 1992; Eisen et al., 2007; Rand et al., 2003; Schulze et al., 1984). The sites in the western range for my study were higher in elevation than eastern sites. However, based on my study, the ranges of elevation at these Marquette County sites, had no effect on *D. variabilis.* Interestingly, the sole site where *I. scapularis* was found had the lowest elevation and closest proximity to Lake Superior, a pattern similar to those of other studies (Amerasinghe et al., 1992; Rand et al., 2003; Schulze et al., 1984).

Ambient temperature, relative humidity, and leaf litter are considered important characteristics in determining tick abundance especially of the microclimate (Harlan & Foster, 1990; Schulze & Jordan, 2005). I measured the mesoclimate temperature and relative humidity at the time of tick collection and neither explained adult *D. variabilis* abundance differences between ranges and among habitat types. However, the soil/leaf litter microclimate is what ticks experience for most of their lives (Dodds et al., 1969; Sonenshine, 1993). Although related, the temperature and relative humidity of mesoclimate change more quickly than the temperature and relative humidity of microclimate. Thus, it is possible for the microclimate conditions to support tick survival when short periods of the mesoclimate do not and thus, may be the better level for these measurements.

My study showed that Marquette County's geologic ranges and habitat types had greater influence on the abundance of *D. variabilis* than the other variables examined. In every habitat examined, there were more ticks in the eastern than in the western half of the county. However, elevation did not explain a significant part of this relationship. A major difference between these regions is the underlying bedrock. Precambrian bedrock such as granite and slate underlying western Marquette County increases the acidity of soils (Albert, 1995; Guerra et al., 2002; Heinrich, 1976; Schwenner, 2007). A coarse comparison of soils showed that soils in the west have lower pH than in the east (Albert, 1995; Schwenner, 2007). Interestingly, portions of the eastern range that have acidic soil are associated with conifer swamps which do not support tick populations (Campbell &

MacKay, 1979; Guerra et al., 2002; Stein et al., 2008). The effect of acidic soils varies among tick species (Guerra et al., 2002; Schwarz et al., 2009). For example, *I. scapularis* has been negatively associated with acidic soils but *I. ricinus* was abundant in moderately acidic soils. Unfortunately, the effect of acidic soils on *D. variabilis* is unknown. A study on the effect of acidic soils on *D. variabilis* survival and development may help explain the reduced tick abundance in the western range of Marquette County.

In both ranges of Marquette County, field habitats had the highest abundance of questing adult *D. variabilis*; this was consistent with the results of other studies (Campbell & MacKay, 1979; Dodds et al., 1969; Petry et al., 2010; Sonenshine & Levy, 1972). Field habitats are highly productive which results in large amounts of litter added to the soil each season, whereas in forests, a greater proportion of primary production is stored as wood (Odum, 1960). More ticks are collected from habitats with ground vegetation and deep leaf litter than from those without (Dodds et al., 1969; Schulze & Jordan, 2005). Field habitats' litter accumulation provides moist soil/leaf litter microclimate. Wooded habitats' well-developed canopies prevent the growth of understory and thereby limit the accumulation of leaf litter (Conlon & Rockett, 1982; Dodds et al., 1969; Schulze & Jordan, 2005). A comparison of 3 types of pine forest, 1 type of hardwood forest, and 1 type deciduous and conifer mix showed that pine litter is denser, breaks down differently, over a shorter period of time and is more acidic compared to deciduous leaf litter (Ottmar & Andreu, 2007). This may help explain the low abundance in mixed and conifer forests. The pH and depth of litter may be regulating the abundance of non-questing ticks in Marquette County habitats.

Collection of Ixodes scapularis

A single adult female *Ixodes scapularis* was collected from the same field in the western range during each sampling year. According to the Center of Disease Control and Prevention, this is a reported population (CDC, 2011).

The collection of two adult *I. scapularis* during my study and subsequent finding of two males and one nymph in 2011 (J. Bird Personal Communication, 2011), suggest that this species has expanded its range into Marquette County. *Ixodes scapularis* prefers coastal lowland areas, and higher humidity in coastal areas increased its survival (Amerasinghe et al., 1992; Rand et al., 2003; Schulze et al., 1984). Interestingly, the *I. scapularis* collected during my study were from the site closest to Lake Superior (approximately 1.3 km) with the lowest elevation compared to the other sites.

Summary and Conclusions

In Marquette County, Michigan, the species of tick that people are most likely to encounter is *D. variabilis.* This exposure risk is higher in the eastern range of Marquette County compared to the western range and in field habitats compared to the other three habitats. Peak questing activity varies year to year but follows a unimodal pattern. It is possible that *I. scapularis* has expanded its range into Big Bay area of western Marquette County.

Future research

Because *D. variabilis* is capable of transmitting diseases to humans, it would be important to investigate whether the tick population in Marquette County carries pathogens. A close study on the effect of soil pH on the survival of all stages of *D. variabilis* may better elucidate the difference in abundance between eastern and western Marquette County. Finally, the difference among habitats could be due to pH levels and leaf litter depths. Investigation into soil characteristics such as depth and pH in Marquette County may better explain the difference in abundance of ticks among habitats. Peak activity of questing ticks varies from year to year, and this could be due to differences in winter and spring from year to year. Further investigation of the microclimate that ticks experience could help explain the timing of peak questing activity and a possible temperature related effect on questing and survivorship of males and females.

Ixodes scapularis has been known to carry the Lyme disease agent and human granulocytic ehrlichiosis agent in neighboring Menominee County (Hamer et al., 2007). The status of the distribution of *I. scapularis* and its prevalence of disease-carrying microbes in Marquette County would be better defined through trapping of mammals and testing for pathogens.

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APPENDICES

APPENDIX A

TABLES

Table 1. Tick species reported in Upper Peninsula of Michigan.

Species	Preferred Habitats	Life Cycle (# Host)[a, b]	Questing Life Stage Expected in Study [b]	Nidicolous or non-nidicolous [b]
Amblyomma americanum	Dense under brush in deciduous forests [a]	3	Larvae, nymph and adult	Non-nidicolous
Dermacentor albipictus	Grassy meadows, deciduous forests [b]	1	None	Non-nidicolous
D. variabilis	Grassy meadows, moist deciduous forests [a, b]	3	Larvae, nymph and adult	Non-nidicolous
Haemaphysalis leporispalustris	Grassy meadows, deciduous forests [a]	3	Larvae, nymph and adult	Non-nidicolous
Ixodes cookei	Grassy meadows, deciduous forests , nests and dens of host [a, b]	3	None	Nidicolous
I. scapularis	High shrub vegetation and deciduous forest [b]	3	Larvae, nymph and adult	Non-nidicolous

[a] Bishop and Trembley, 1945
[b] Sonenshine, 1993

Table 2: Average temperature and precipitation data: 30 year, 2008 and 2009. Averages for winter (December, January, Febuary), spring (March, April, May) and summer (June, July, and August) were calculated using monthly averages. (30-year averages from NCDC/NESDIS/NOAA, 2002; season averages during 2008 and 2009 from NWS, 2011.)

	Winter T (□C)	Winter ppt (cm)	Spring T (□C)	Spring ppt (cm)	Summer T (□C)	Summer ppt (cm)
30-year average	-9.7	5.8	2.7	7.6	16.7	8.3
2008	-9.3	1.23	1.4	8.9	17	5.6
2009	-10.3	6.9	2.7	5.8	15	7.2

Table 3: Number of male and female *Dermacentor variabilis* collected from four habitats in eastern and western ranges of Marquette County, MI during 2008 and 2009.

	2008				2009				
	East		West		East		West		
	Male	Female	Male	Female	Male	Female	Male	Female	Sum
Field	141	178	74	70	236	243	63	52	1057
Hardwood	72	89	29	28	55	49	17	10	349
Mix	32	40	10	16	12	19	4	6	139
Conifer	0	0	0	0	0	2	0	0	2
Sum	245	307	113	114	303	313	84	68	
Overall Total	552		227		616		152		1547

APPENDIX B

FIGURES

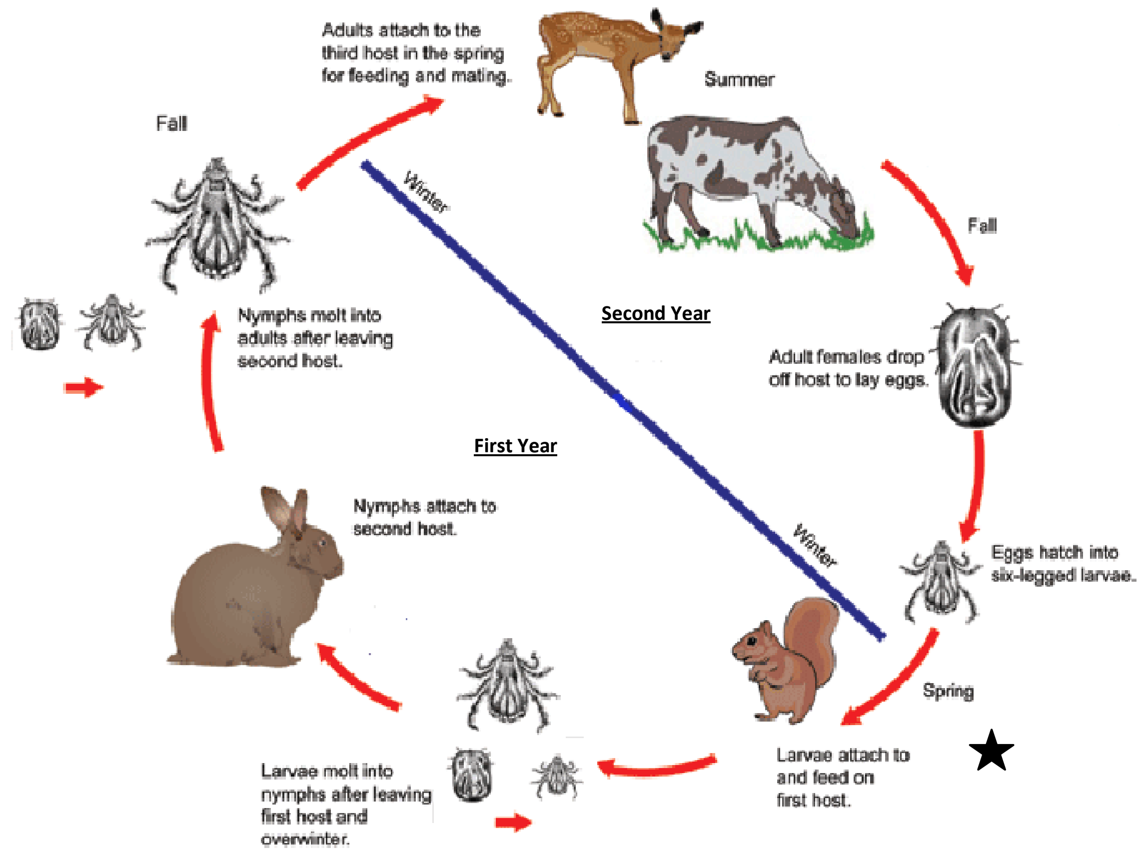


Figure 1. Two-year life cycle of ixodid ticks. Star indicates starting point for the life cycle. During the first year, in spring overwintered larvae emerge, quest and molt into nymphs. Nymphs will quest again and molt into adults after leaving second host. In late summer into fall, adults enter diapause and will overwinter, emerging in the second year during the spring to quest for a final host, mate, and engorged females will lay eggs and die. The eggs will hatch into larvae and overwinter as larvae to emerge the following spring, completing the cycle (CDC, Life cycle of 3-host ticks, 2010).

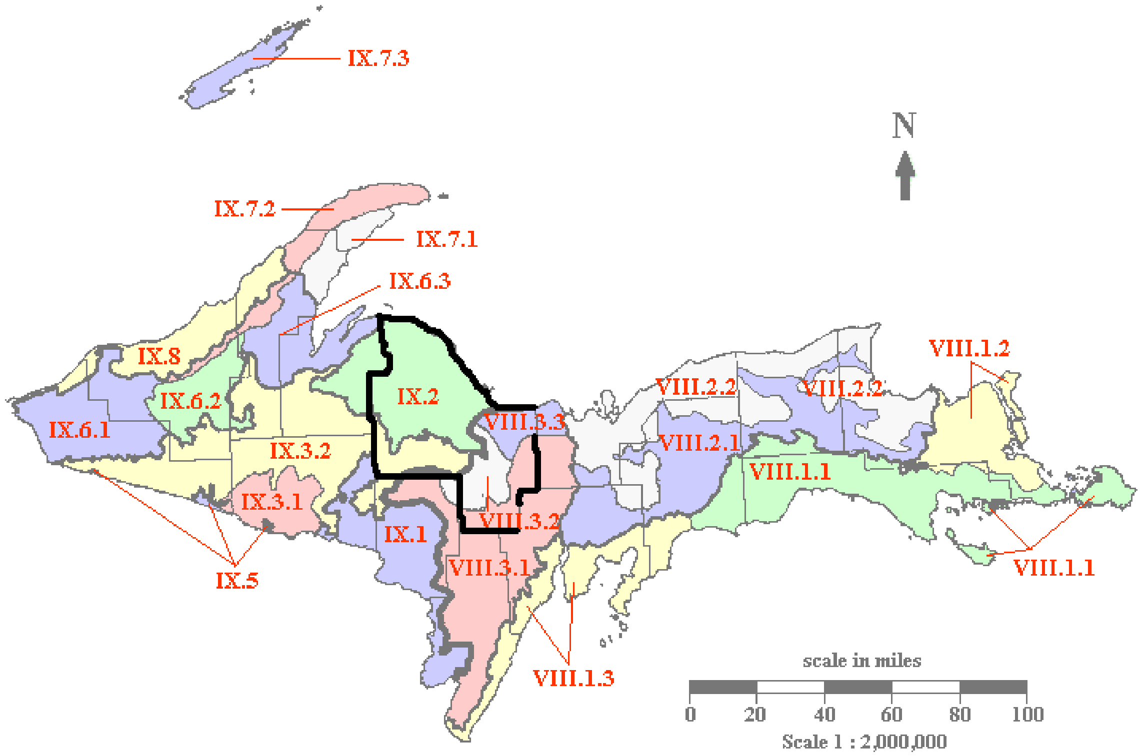


Figure 2. Regional Landscape Ecosystem of Michigan's Upper Peninsula (Albert, 1995). Marquette County, Michigan's border is outlined in dark black. The heavy grey line running north to south is the geologic division of the Upper Peninsula, creating a western and eastern range.

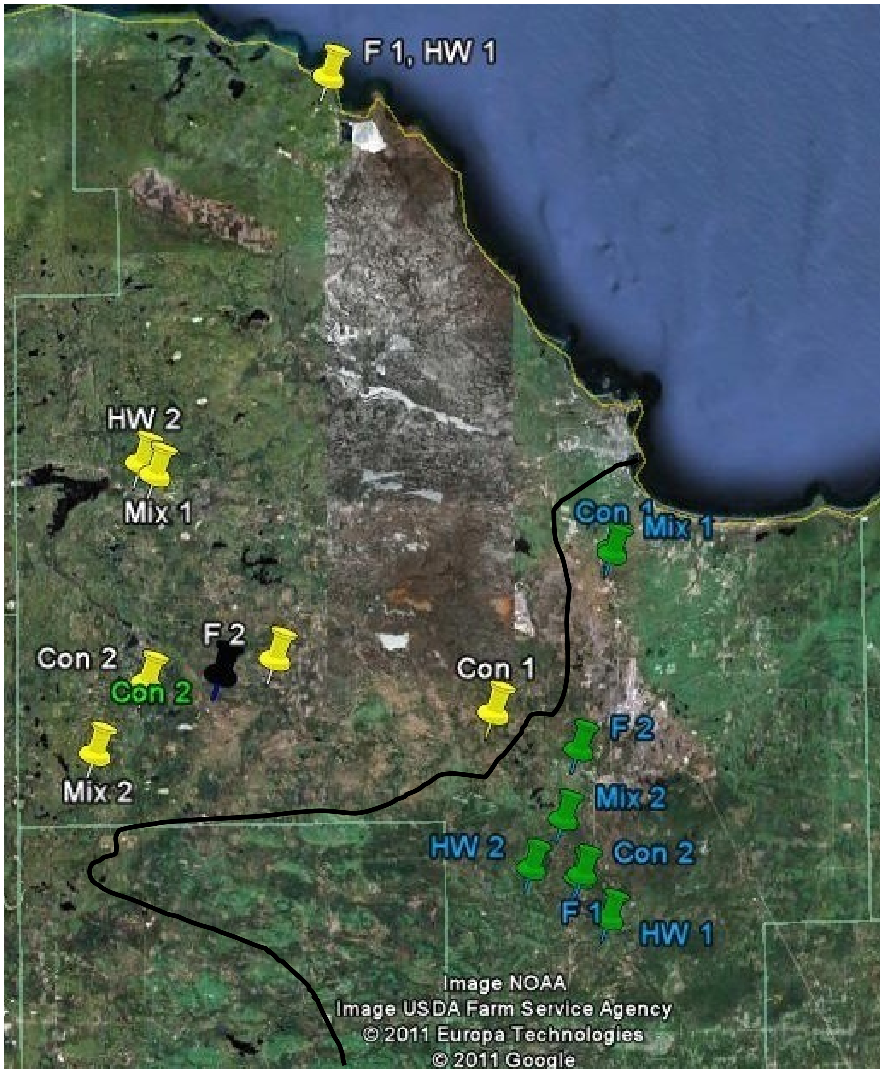


Figure 3. Sampling sites used during 2008 and 2009 survey. All sites remained the same for both seasons except for one conifer site in 2009, indicated by the black pushpin labeled "Con 2". Heavy black line indicates the natural geologic division (Google Earth, Imagery Date June 1, 2005).

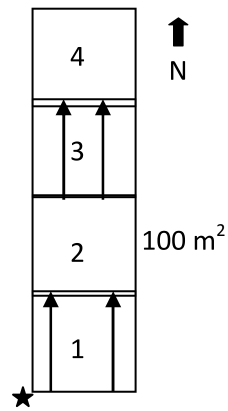


Figure 4. Orientation of site plots. Each square represents the 100 m^2 plot of the desired habitat type. The star indicates the southwestern corner of the plot. Four 100 meter transects were randomly chosen and walked for a total of 400 meters each sampling period.

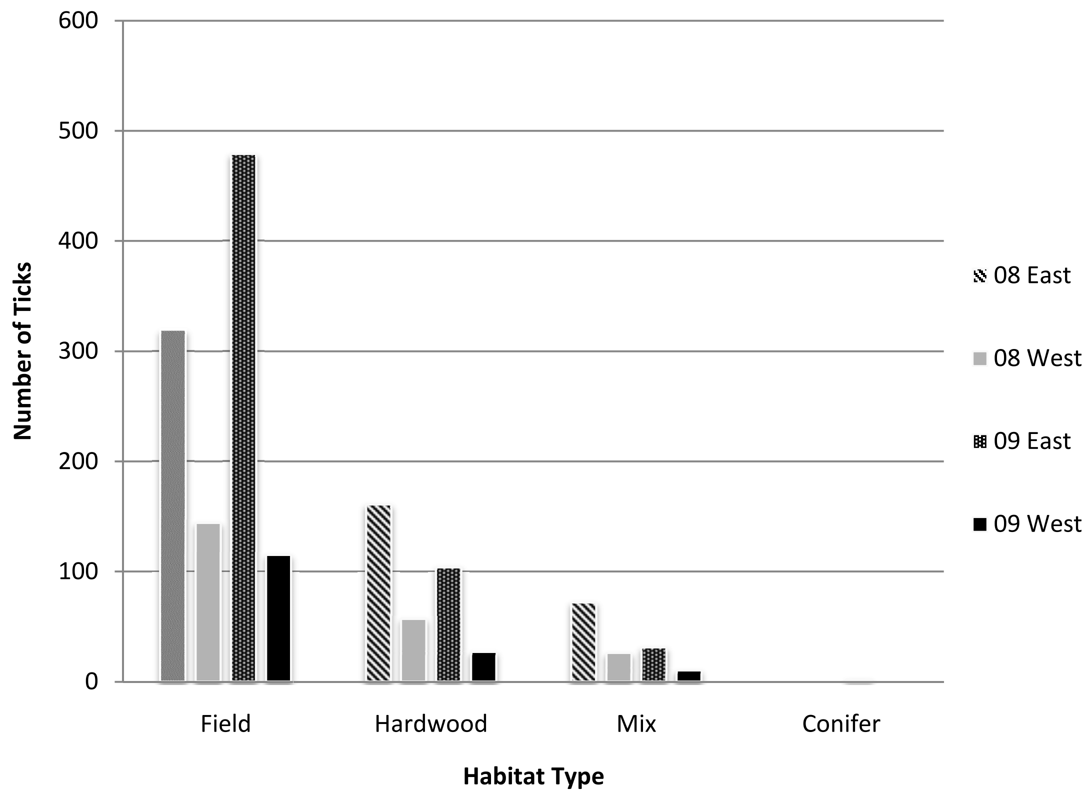


Figure 5. Total number of ticks collected in four dominant habitat types (field, hardwood, mix and conifer) in eastern and western Marquette County during 2008 and 2009 sampling seasons. The conifer habitat only had two ticks collected from the east in 2009.

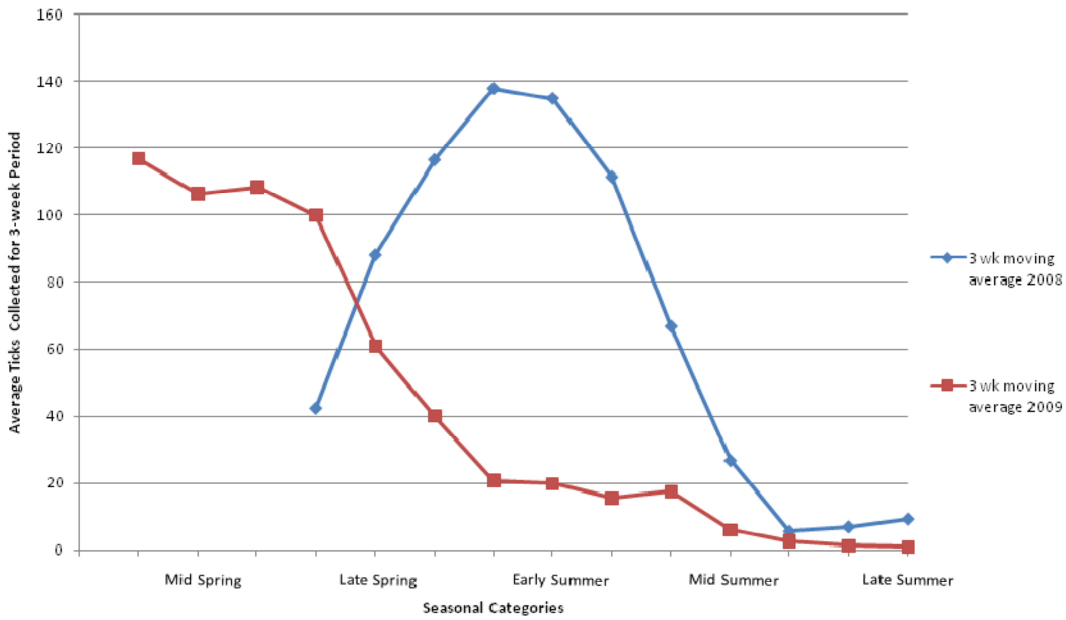


Figure 6. Temporal patterns of ticks over the two sampling periods. Both seasons indicated that *Dermacentor variabilis* population in Marquette County, Michigan is unimodal although different patterns are visible.

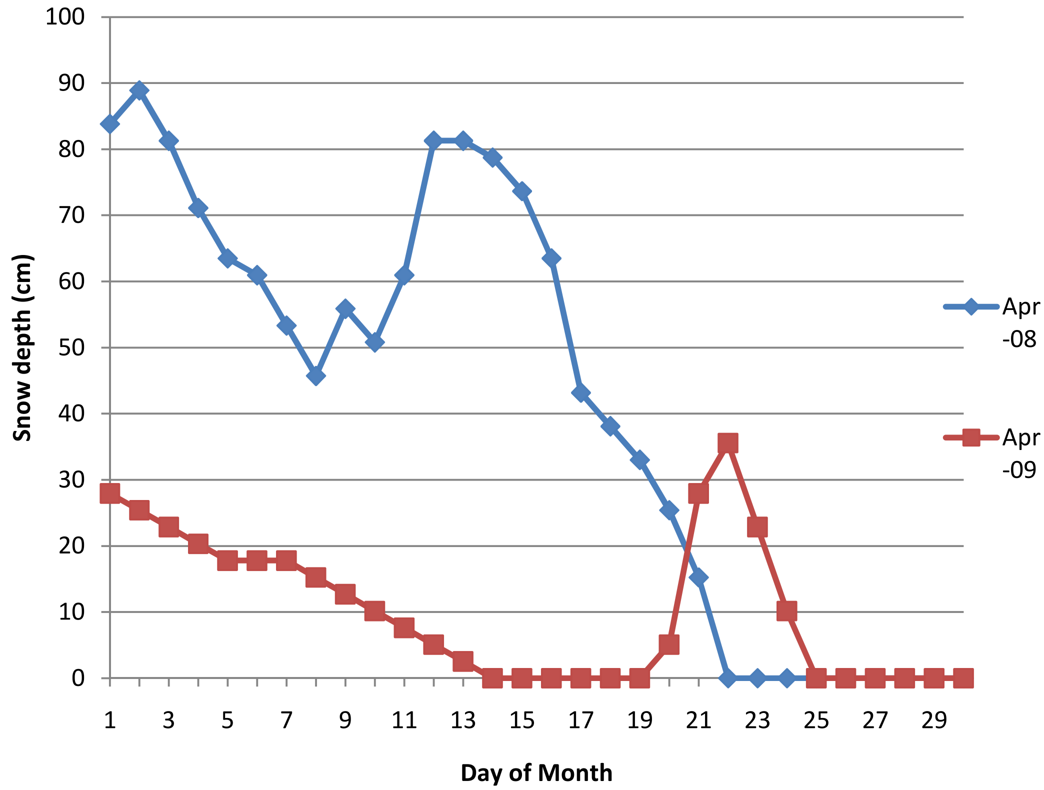


Figure 7. Snow depth during April 2008 and 2009. There was 15.2 cm of snow until April 22, 2008 and until April 8, 2009. There was some snow fall April 20-April 24, 2009 (NWS, 2011).

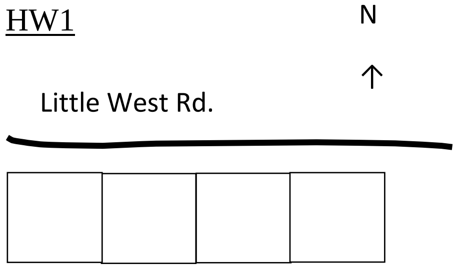
APPENCIX C

SITE LAYOUTS

Site layouts for future long term studies.

Eastern Range

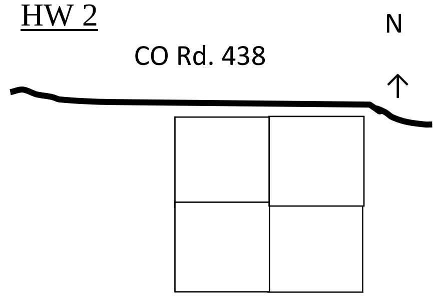
HW1



T.43N. – R.25W. Sec 4SE

Coordinates: 46.145, -87.433

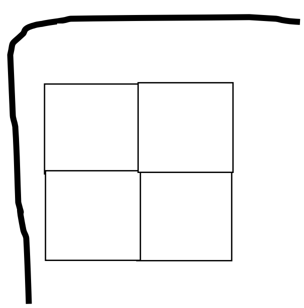
HW 2



T.44N. – R.26W. Sec26NE

Coordinates: 46.187, -87.523

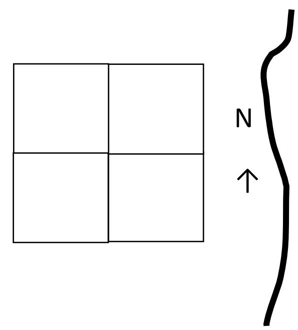
Mix 1



T.47N. – R.25W. Sec 27SC

Coordinates: 46.447, -87.421

Mix 2

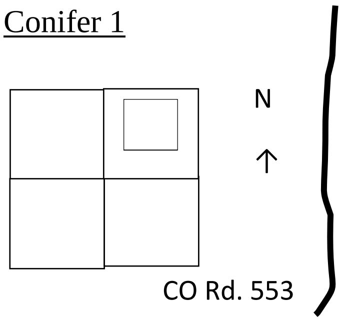


T.44N – R.25W Sec WC

Coordinates: 46.227, -87.484

Eastern Range

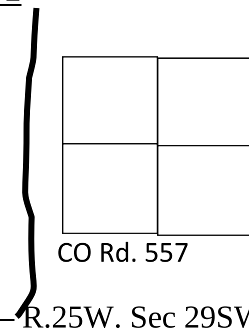
Conifer 1



T.47N. – R.25W. Sec 34C

Coordinates: 46.443, -87.421

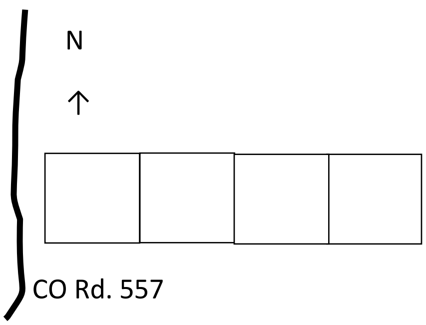
Conifer 2



T.44N. – R.25W. Sec 29SW

Coordinates: 46.182, -87.463

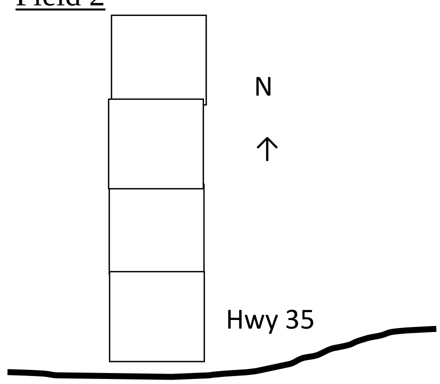
Field 1



T.44N. – R.25W. Sec 29SW

Coordinates: 46.179, -87.467

Field 2

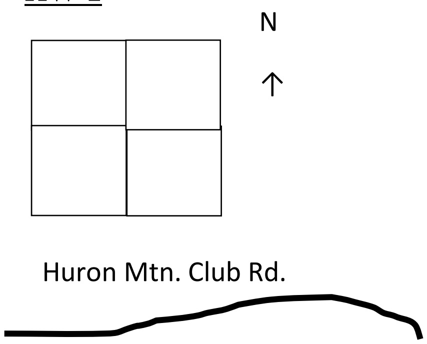


T.45N. – R. 25W. Sec 20C

Coordinates: 46.283, -87.465

Western Range

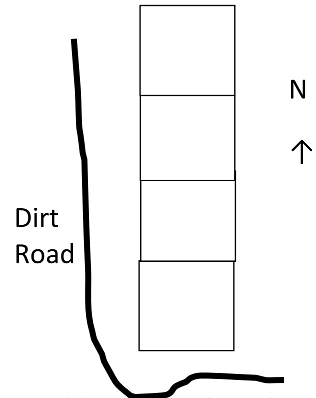
<u>HW 1</u>



T.51N. - R. 27 W Sec 8NE

Coordinates: 46.833, -87.750

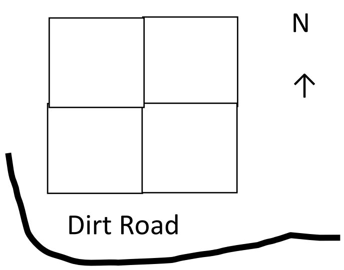
<u>HW 2</u>



T.48N. – R.29.W. Sec 31NC

Coordinates: 46.520, -87.977

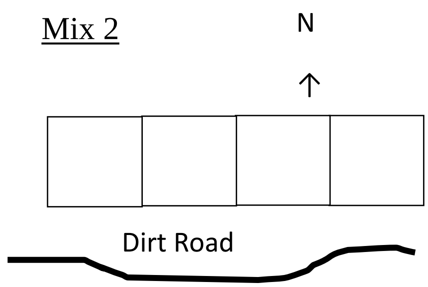
<u>Mix 1</u>



T.48N. – R. 29.W Sec 32C

Coordinates: 46.510, -87.961

<u>Mix 2</u>

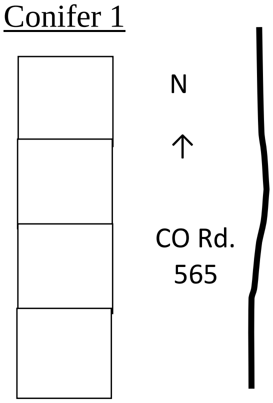


T.45N. – R.30W. Sec22 NE

Coordinates: 46.284, -88.036

Western Range

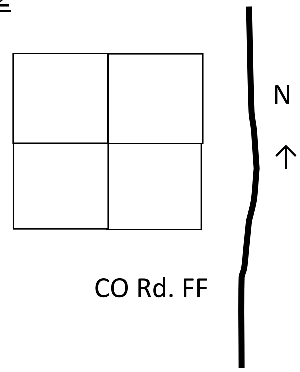
Conifer 1



T.46N. R.26W. Sec 31NW

Coordinates: 46.313, -87.566

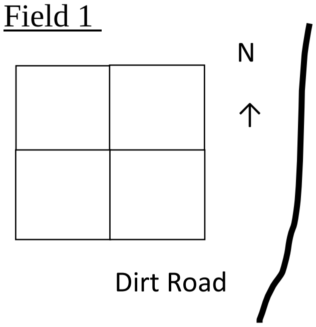
Conifer 2



T. 46.N. – R.29W. Sec 36NC

Coordinates: 46.348, -87.886

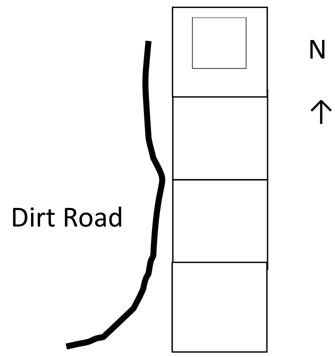
Field 1



T.51N. – R.27W. Sec 8NE

Coordinates: 46.833, -87.750

Field 2



T.46N. – R.28W. Sec 21SW

Coordinates: 46.359, -87.822